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An Ecofriendly Method for the Synthesis of Zinc oxide Nanoparticles using *Lawsonia inermis* L. Aqueous Extracts

M.S. Shekhawat*[1], C.P. Ravindran[1], M. Manokari[2]

[1]Biotechnology Lab. Mahatma Gandhi Govt. Arts College, Mahe, Pondicherry, India.
[2]Biotechnology Unit, K.M. Centre for Post Graduate Studies, Pondicherry, India.
smahipal3@gmail.com

ABSTRACT
The biological synthesis of Zinc oxide nanoparticles using *Lawsonia inermis* L. plant extracts was studied. Development of green, nontoxic and clean techniques for the synthesis of Zinc oxide nanoparticles (ZnNPs) has attracted increasing attention in recent years. Henna is known to be a traditional product with religious associations, and widely used over the centuries for medical and cosmetic purposes. Leaves, stem and root aqueous extracts of *Lawsonia inermis* were used to synthesize the nanoparticles. Zinc Nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) solution was used as precursor solution. The synthesized nanoparticles were confirmed by color changes from brown to pale green and characterized by UV-Visible spectrophotometer. Absorption peaks in between 296 nm to 302 nm were observed to confirm the presence of Zinc oxide nanoparticles in the solution.

Key words: Biogenesis, Zinc oxide nanoparticles, *Lawsonia inermis*, characterization.

INTRODUCTION
The nanoparticles of metal oxides are the important components of household products. The application of nanoparticles in cosmetics and medical field is also continuously increasing day by day [1]. Zinc oxide (ZnO) is an inorganic compound known as white powdered Zincite. It has the property of good conductivity and heat capacity [2]. ZnO in nano size is a key component in cosmetics, dye sensitized cell, plastic additives and electronics because it blocks UV rays. Thin films of nano ZnO play an important role in drug delivery and other medicinal applications [3].

In this study we aimed to develop a cost effective, nontoxic, ecofriendly biosynthesis protocol of Zinc oxide nanoparticles using *Lawsonia inermis* L. It is an important medicinal plant belongs to the family Lythraceae (Fig. 1). It is known as mignonette tree, henna tree, mehandi etc. and distributed in dry tropical and subtropical zones. Henna is known to be a traditional product with religious associations, and widely used over the centuries for medical and cosmetic purposes in Africa, Asia, the Middle East and many other parts of the world [4].

This plant has been described in the Indian system of traditional medicine for the treatment of epilepsy and jaundice, dyeing grey hairs and malignant ulcers [5]. According to Ayurvedic Pharmacopoeia of India *Lawsonia inermis* has been used in dysuria, bleeding disorder, prurigo and other obstinate skin diseases, headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilitis, sores, amenorrhoea, scabies, and spleen diseases [6].

Fig. 1. *Lawsonia inermis* in natural habitat.

Marimuthu et al, [7] synthesized silver nanoparticles using *L. inermis* extract. Spherical shaped silver nanoparticles (AgNPs) were also synthesized by Venkata Subbaiah and Savithramma [8] using the aqueous axtract of henna. Arpita et al, [9] tested these AgNPs efficacy against some pathogens.

Aqueous extracts of various parts of *Lawsonia inermis* were used for the first time as a reducing and capping agent with Zinc Nitrate hexahydrate as a precursor to synthesize Zinc oxide nanoparticles in this study.

MATERIALS AND METHODS
Lawsonia inermis L. is also known as multipotent plant in India. The plant materials were collected from the coastal area of Pondicherry, India. The plants were identified with the help of 'The Flora of Presidency of Madras'[10]. Fresh, green and mature

leaves, stem and roots were collected during the months of January to May 2014. The materials were thoroughly washed with distilled water and finely cut in small pieces (Figs.2-4A and B.)

The plant extracts (broth solutions) were prepared by using 5gm of washed and cut leaves, stem and roots in a 250ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5min in water bath. The herbal aqueous extract was collected in separate conical flasks by the standard filtration method and stored at 4ºC in a refrigerator.

1mM Zinc nitrate solution was prepared using Zinc Nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) (Merck, Mumbai, India) and stored in brown colored bottle at 4ºC for further use. For the synthesis of Zinc oxide nanoparticles, three boiling tubes were taken, one containing 10ml of 1mM Zinc nitrate solution as control and the second one containing 10ml of plant extract and third containing 9ml of 1mM Zinc nitrate solution and 1ml of plant extracts as test solution (Figs. 2-4C).

The Zinc nanoparticles synthesized using aqueous extracts of *L. inermis* were centrifuged at 5000 rpm for 15 min in order to obtain the pellet which is used for further study. Supernatant is discarded and the pellet is dissolved in deionized water. The zinc nanoparticles were confirmed and characterized by UV-Visible spectrophotometer (Systronics Double Beam Spectrophotometer, (Model 2202, Systronics Ltd. India). The UV-Vis absorption spectra of the zinc colloids were recorded by using wave length scan between 200nm and 700nm.

RESULTS AND DISCUSSION

Extracts from plants may act as reducing and capping agents in metallic nanoparticles synthesis. The reduction of metallic ions by combinations of biomolecules found in these extracts (e.g. enzymes/proteins, amino acids, polysaccharides, vitamins etc.) is environmentally benign, yet chemically complex.

The green synthesis of Zinc oxide nanoparticles through aqueous extracts of *Lawsonia inermis* was carried out in present investigation. The color was changed in the cell free extract when challenged with 1mM Zinc nitrate solution from brown to pale yellow (Figs. 2-4C) within 10 min and attained maximum intensity after 14 hrs with intensity increasing during the period of incubation indicative of the formation of nanoparticle. Control showed no change in color of the cell filtrates when incubated under same conditions.

Fig. 2A. Fresh leaves, 2B. Leaves in small pieces, 2C. Zinc Nitrate solution, aqueous extract of leaves and the mixture.
3A and B. Stem cuttings, 3C. Zinc Nitrate solution, aqueous extract of stem and the mixture.
4A and B. Root cuttings, 4C. Zinc Nitrate solution, aqueous extract of roots and the mixture.

The presence of quinones in henna plant acts as dyeing agent. The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone

hydroquinone pair is very important in many biological systems [11].

Lawsonia inermis extract was used to synthesize silver nanoparticles by various researchers [7, 8] and characterized with the UV spectrophotometer. Arpita et al, [9] tested these AgNPs efficacy against *Candida albicans*, *Microsporum canis*, *Propionibacterium acne* and *Trichophyton mentagrophyte*.

Biogenesis of plant extract mediated ZnO synthesis would be fulfilled by the characterization of UV-Vis spectrometer by monitoring the changes in the light scattering when passed through ZnO suspensions as a function of time. The leaf and root extracts shows absorption range at 302nm and the root shows at 296nm (Figs. 5A, B, C and D). The root and leaves contain almost same phytochemicals (varies at quantity level) to attribute the red colour [12]. The presence of same types of phytochemicals represents the absorption peak at 302nm.

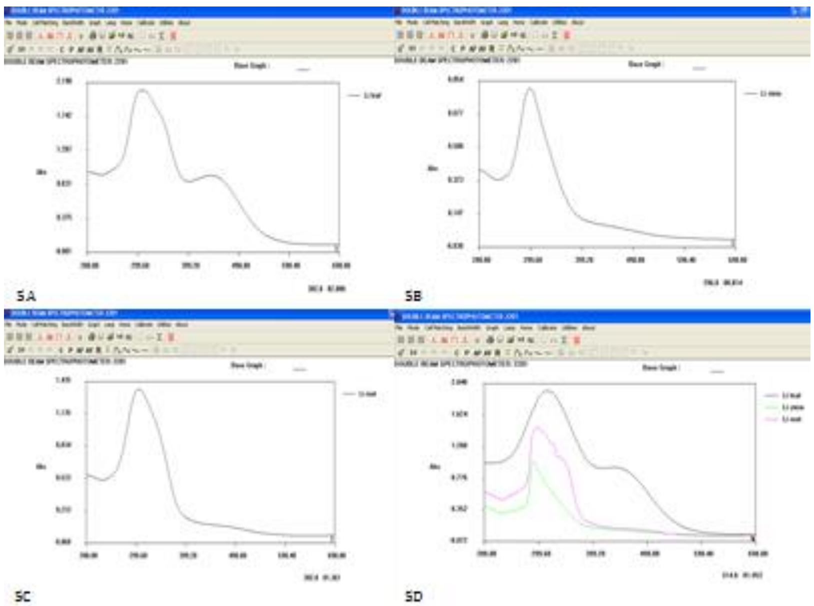

Fig. 5A. Spectrophotometric absorbance peak of leaf reaction mixture.
Fig. 5B. Spectrophotometric absorbance peak of root reaction mixture.
Fig. 5C. Spectrophotometric absorbance peak of stem reaction mixture.
Fig. 5D. Combined absorbance peaks of leaf, root and stem reaction mixtures.

Various other reports also support our findings. Plant extract mediated biosynthesis, characterization and applications of Zinc oxide nanoparticles have been investigated in number of plants like, *Calotropis gigantea* [13], *C. procera* [14], *Passiflora foetida* [15], *Morinda pubescens* [16], *Acalypa indica* [17] etc. This protocol could be used in the commercial preparation of ZnNPs using various plant part extracts and further used in the field of agriculture (as liquid nano-bio- fertilizers) and medicine.

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